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Mutations in Human Gonadotropin and Gonadotropin-Receptor Genes

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This short review provides an update on the new information that has become available in the recent years about mutations and polymorphisms in the genes for gonadotropins and their receptors. Combining the types and locations of the mutations, their phenotypic effects, and the recently emerged information about the crystal structure of these molecules is providing us with increasingly detailed picture about the structure–function relationships of gonadotropin action.

Key Words: Follicle-stimulating hormone; gonadotropins; gonadotropin receptors; luteinizing hormone; mutation; polymorphism.

Introduction

About 5 yr ago, the authors of this review published a comprehensive overview of mutations in gonadotropin subunit and receptor genes and their pathophysiological consequences *(1)*. The knowledge in this field has expanded considerably during the past years, and our intention is now to present an update about the knowledge in this field that has accumulated since the writing of our previous review. We start with a short introduction into the general principles of pathophysiological consequences of mutations and polymorphisms affecting gonadotropin action, and move on thereafter to the recent advances in the field. In particular, we will discuss the new polymorphisms and mutations that have been detected in the genes for the *luteinizing hormone (LH), follicle-stimulating hormone (FSH)*, and their *receptors*. For a comprehensive overview of the topic until 2000 we refer to ref. *1*, which contains extensive tables and figures specifying most of the mutations and variants of *LH, FSH*, and their *receptor* genes.

The differences between polymorphic and mutated alleles of a gene are not always completely clear because scientists from different backgrounds use these terms with different meanings. Here we will use the genetic definition that calls a DNA variant a polymorphism when it occurs at a frequency of higher than 1%. This definition is neutral and avoids the functional connotation involved in the word "mutation." However, it does not imply that mutations always have negative effects, or that polymorphisms have no functional effects.

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Functional Consequences of Mutations and Polymorphisms Affecting Gonadotropin Action

Gonadotropin Subunits

Up to now, only few mutations are known in the gonadotropin subunit genes (Fig. 1). Two subjects with LHβ and seven with FSHβ inactivation have been described in the literature *(1–3)*. Conspicuously, no germ line mutations in the *common-α subunit* or *hCGβ subunits* are known. The reason may be that such mutations would inactivate chorionic gonadotropin (hCG), which may be embryo-lethal, or even incompatible with implantation.

The crystal structures of deglycosylated hCG and FSH are known *(4,5)*, and, as expected, they are very similar. The crystal structure revealed that the two gonadotropins belong to the superfamily of cystine knot growth factors, characterized by a cluster of three cystine disulfide bonds in each subunit. Similar folding is found in some protein growth factors, such as nerve growth factor, transforming growth factor-β, and platelet-derived growth factor-β. Although the α- and β-subunits show no amino acid sequence similarity, their three-dimensional structures are remarkably similar, including two β-hairpin loops on one side and a single loop on the opposite side of the cystine knot structure. The β-hairpins are stabilized by additional disulfide bridges. The two subunits are associated in a head-to-tail orientation, forming an elongated, slightly curved structure. The dimeric molecule is stabilized by a "seatbelt" structure formed by the C-terminal amino acids of the β-subunit wrapped around the α-subunit and stabilized by one of the disulfide bonds. This extraordinary structure is essential both for the association of the subunits through non-covalent interactions and for receptor binding. Extensive studies on site-directed mutagenesis have unraveled the role of a number of amino acids in the tertiary structure of this subunit, including the structures necessary for glycosylation, proper folding, heter-

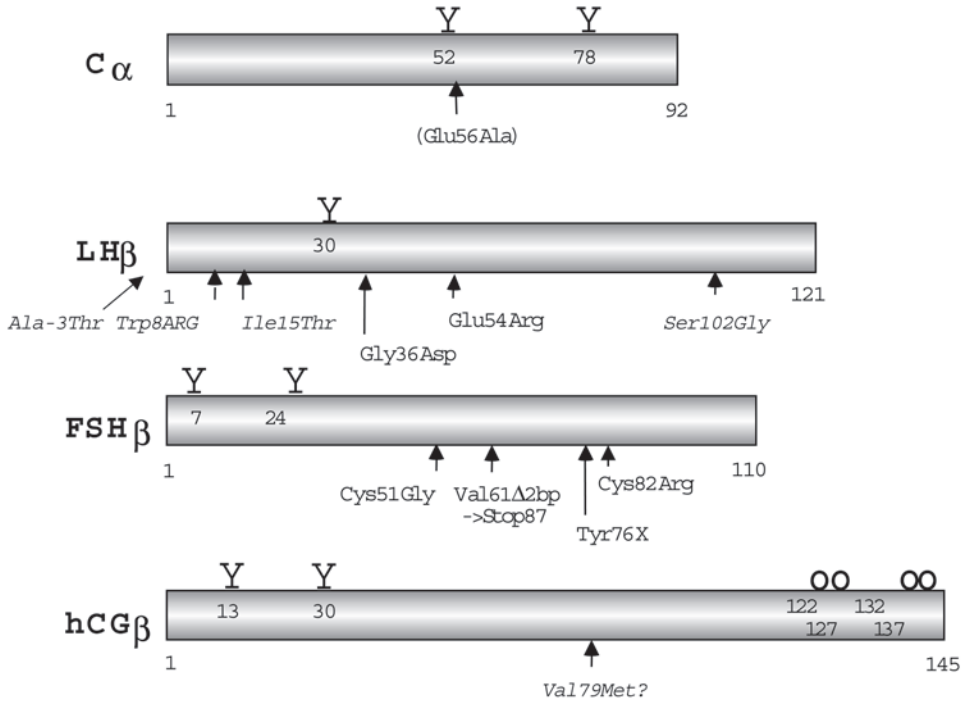

Fig. 1. The currently known mutations and amino-acid-altering polymorphisms in the *common α-subunit*, *LH*β, *FSH*β, and *hCG*β genes. For references and further details, see text. The alterations in *italic* are polymorphisms.

odimerization with the β-subunit and receptor binding, and signal transduction of the dimeric hormone.

Gonadotropin Receptors

The LH receptor together with its homologs, the receptors for FSH and TSH, constitute the glycoprotein hormone receptors, a subfamily of the large group of G protein–coupled receptors (GPCRs). The LH receptor has both LH and hCG as its ligands, whereas FSH and TSH receptors are activated only by their single cognate ligands. Glycoprotein hormone receptors are characterized by a large N-terminal extracellular domain (ECD; 359–414 amino acid residues) and a rhodopsin-like transmembrane (TM) domain *(1,6–8)*. The structural organization of the TM region of all GPCRs has been assigned using the structure of bovine rhodopsin as a template *(9)*. While the TMs of individual glycoprotein hormone receptors are functionally interchangeable and display high sequence homology (approx 70%), the ECDs are less similar (approx 40%) being responsible for selective hormone recognition and binding *(10–12)*.

Structures of the ECDs of the glycoprotein hormone receptors were not available until very recently (see below), but their sequences were suggested to contain nine leucine-rich repeats (LRR) flanked by N- and C-terminal cysteine-rich regions. LRR motifs have been recognized in a large number of distinct proteins *(13)*. The existing crystal structure of ribonuclease inhibitor (RI) showed that its LRRs are in a horseshoe-like conformation, in which each LRR (approx 20–24 amino acid residues) is organized into a short β-strand connected to a parallel α-helical segment *(14)*. The consecutive β-strands organize themselves as a parallel β-sheet, forming a concave surface to which the ligand (i.e., RNase) binds using multiple contact points, whereas the helical segments are aligned to form the outer convex side of the RI structure. Based on the RI structure, the LRR portions of the ectodomains of the TSH and LH receptors have been modeled *(15–17)*. Binding of LH or hCG to the LH receptor is predicted to involve multiple contact points with the concave β-sheet of its curved ECD.

The report of crystal structure of FSH complex with the ECD of the FSH receptor has corrected and further refined the existing models *(18)*. The main features are that the curvature of the hormone-binding concave β-sheet is less strong. The β-sheet contains nine parallel strands and a tenth that is contributed by the N-terminal cystine repeat *(18)*. FSH binds with its long axis perpendicular to the FSH receptor tube formed by the concave β-sheet. In addition, detailed information on the identity of the amino acids that are involved in the interaction between FSH and its receptor is revealed by the analysis of this structure. The seat belt structure mentioned above is directly involved in hormone–receptor interaction *(18)*. Another novel feature revealed by this recent study was that the receptor apparently dimerizes following ligand binding.

Mutations and Polymorphisms in Human Luteinizing Hormone and its Receptor

LHβ Subunit

Mutations

Mutations in the *LH*β gene (Fig. 1) have proven to be very rare, consistent with their expected deleterious effects on

fertility. Very recently, the second report of a homozygous carrier of a missense inactivating mutation in the *LH*β gene was published *(2)*. The 30-yr-old man presented with hypogonadism with absence of circulating LH. A testis biopsy showed the typical aspect associated with absence of LH signaling, as is also seen in patients with inactivating *LHR* mutations, i.e., hypoplastic tubules with greatly reduced spermatogenesis and some hypoplastic Leydig cells. DNA sequencing revealed a Gly36Asp amino acid change in exon 2 of the *LH*β gene. This mutant *LH*β was expressed in vitro together with the common-α subunit, and showed absence of α/β heterodimerization, whereas the wild-type LHβ protein was able to do so *(2)*, resulting in the absence of circulating LH. The Gly36Asp in the LHβ subunit disrupts a five-amino-acid motif that allows the formation of a cystine knot. The cystine knot consists of an eight-amino-acid ring through which an intrasubunit disulfide bond is formed. Gly36 is essential to allow the disulfide bond to be formed. This effect of the mutation is different from the other published inactivating *LH*β mutation *(19)*. In this case the Gln 54Arg abolished interaction with LH receptor, resulting in delayed puberty and absent testosterone production and spermatogenesis in the patient *(19)*. Both *LH*β mutations illustrate the role of LH during sex differentiation. Apparently, LH is not necessary for male differentiation before birth, because both patients had a male phenotype and descended testes. The fetal activation of Leydig cell proliferation, differentiation, and testosterone production is taken care of by placental hCG, allowing these patients to undergo fetal male sex differentiation and insulin-like factor 3–mediated testis descent.

Polymorphisms

Four different polymorphisms have been described in the *LH*β gene (Fig. 1). The best investigated *LH*β subunit polymorphism is the T82C/T104C combination that results in two amino acid changes: Thr8Arg/Ile15Thr *(20,21)*. This combination of polymorphisms is in complete linkage disequilibrium and has been named variant (V) LH. The other two polymorphisms, Ser102Gly *(22)* and Ala-3Thr *(23)*, have been studied to a lesser extent (see below).

The V-LH polymorphism was first identified when it was recognized that a certain monoclonal antibody that was used in an immunofluorometric assay for serum LH did not recognize the hormone in a healthy woman who was homozygous for the *V-LH*β allele, whereas the bioactivity of her LH was normal and in accordance with her normal fertility *(24)*. Subsequently, several studies were conducted to investigate possible effects of the two amino acid changes on LH function and to identify possible associations with disease endpoints (reviewed in ref. *1*). V-LH is found worldwide at highly variable frequency in cohorts studied from different countries and different ethnic groups. Most recent association studies of the *V-LH*β polymorphism were done using relatively small cohorts or were case reports *(25–27)*.

No association of the *V-LH*β allele with infertility was found in cohorts of 95 male infertility patients *(28)*, in a comparison of 145 infertile with 200 fertile men *(29)*, or in association studies of several endocrine-related cancers *(30,31)*. Nevertheless, V-LH appears to have functional effects, because in a study on 40 healthy Japanese women *V-LH*β carriers appeared to respond to a GnRH challenge with higher maximal LH response *(32)*. Further evidence for the functional differences of V-LH from normal LH was obtained by comparing the in vitro and in vivo behavior of recombinant forms of the two hormones. V-LH displayed higher biopotency in vitro, whereas its half-life in circulation was shorter than that of normal LH *(33)*. Moreover, the carbohydrate side chain composition of V-LH was clearly different, suggesting different pathways in its intracellular processing.

In vitro analysis of the Gly102Ser LH variant revealed that it appeared to be equivalent to the LHβ *(34)*. The frequency of the Gly102Ser LHβ polymorphism appears to be low, and in cohorts from Finland, India, Denmark, and Rwanda the polymorphism was absent *(34)*. In Singaporean Chinese subjects, Gly102Ser was more often detected in infertile men (5 out of 145) than in the fertile control group ($n = 200$) *(29)*, whereas other studies (422 Korean individuals) failed to find any carriers of this polymorphism *(28,35)*.

In a search for other polymorphic *LH*β alleles, Jiang and colleagues found an amino acid change located three residues before the signal peptide cleavage site, Ala-3Thr *(23)*. In vitro studies showed that the Ala-3Thr variant has slightly different signal transduction properties as compared to the wild-type LH, although the mature proteins are essentially identical with respect to their amino acid composition. Wild-type LH was more potent in stimulating cAMP in treated cells, whereas the Ala-3Thr variant was more potent in stimulating the PI cycle *(23)*.

As stated before, no mutations in *hCG*β subunit have been detected, possibly owing to their potentially deleterious effects on pregnancy. An interesting polymorphism in *hCG*β, a missense G to A mutation, resulting in Val79Met change, was discovered several years ago in the mid-west of the US *(36)*. The frequency of the polymorphism was rather high, 4.2%, and it suppressed the α/β dimerizaton of recombinant hCG protein. However, when the same polymorphism was searched for in 580 DNA samples from four European populations, not a single case was found *(37)*. The real frequency and significance of this polymorphism thus remains open.

LH Receptor

As reviewed previously *(1)*, LH receptor mutations (Fig. 2) come in two flavors, activating, causing precocious puberty in boys, and inactivating, which disrupts sex differentiation in men and causes anovulation in women. In the following we will review the recent new mutations that have been described in the LH-receptor gene.

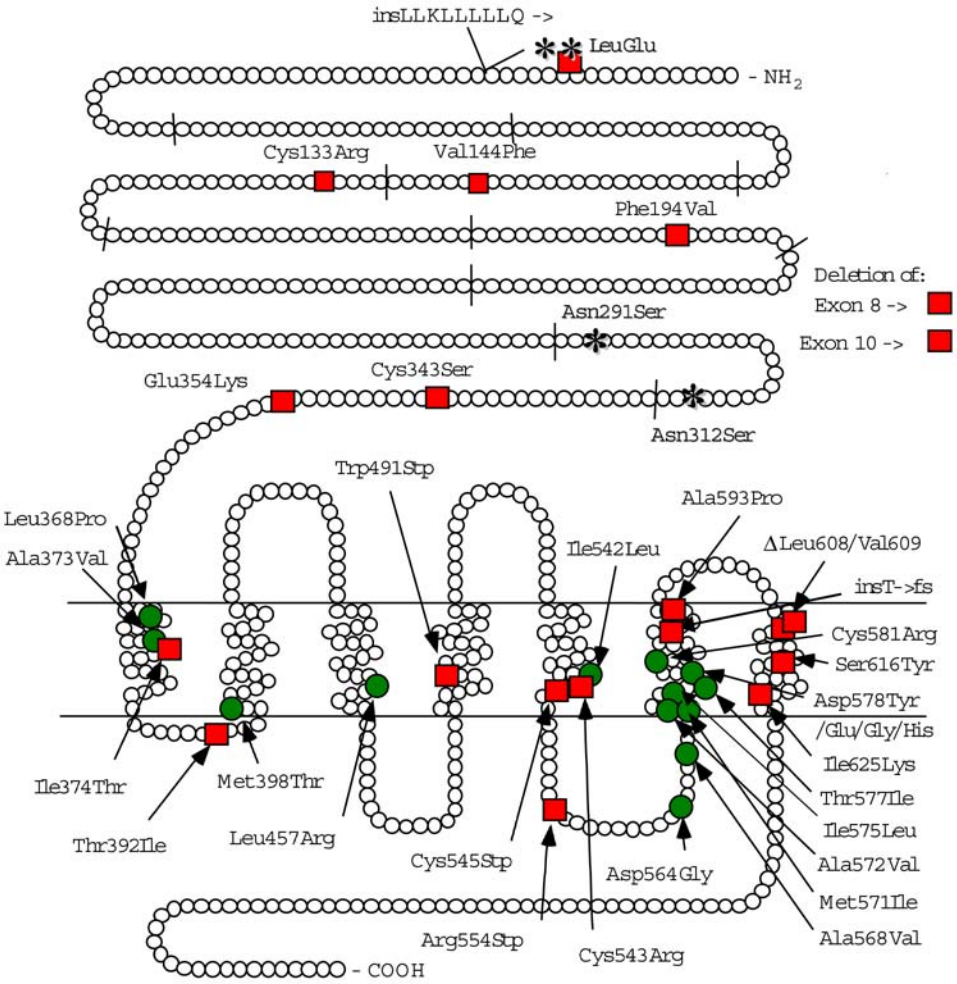

Fig. 2. The currently known mutations and amino-acid-altering polymorphisms in the human LH-receptor gene. The circles depict the activating, the squares inactivation mutations, and the asterisks the polymorphisms. ins = insertion; fs = frameshift.

Activating LHR Mutations

One new mutation that causes constitutive activation of the LH receptor has been identified since our last review *(1)*, a Leu368Pro missense mutation located in the first transmembrane helix of the transmembrane domain *(38)*. This mutation was found in two Brazilian brothers who showed typical precocious puberty at an early age (2 and 3 yr). Their mother also carried the mutation, showing that constitutive activation of the LH receptor does not interfere with female fertility. In vitro expression of the mutant LH receptor showed increased cAMP production without addition of the ligand hCG. At higher hCG concentrations the receptor responded with further increase in cAMP, however never reaching the levels attained at maximal response in the non-mutant LH receptor. The missense Asp578His mutation deserves special attention; it causes Leydig cells to undergo transformation and their increased growth results in adenoma formation *(39)*. Interestingly, this mutation appears to occur somatically, i.e., it is found only in the Leydig tumors themselves, but not in the germ line. Similar case reports have been published of the same Asp578His mutation *(40,41)*. However, two studies on sex cord tumors failed to reveal LH-receptor mutations, indicating that not all Leydig cell adenomas result from somatic *de novo* mutations in the LH-receptor gene *(42,43)*.

Another LH-receptor mutation in TM2, Met398Thr, has been reported in case reports *(44,45)*. This mutation is of special interest, because it shows incomplete penetrance, i.e., not all male family members that are carriers of the mutation show the precocious puberty phenotype *(46)*. It will be of interest to study these families in more detail, because the incomplete penetrance may indicate interaction with a modifier gene that silences the activating effects of the amino acid change in the LH receptor.

Inactivating LHR Mutations

As may be expected from the nature of inactivating mutations, these gene changes can take many forms *(1)*: large partial gene deletions to smaller deletions of just two amino acids and nonsense mutations resulting in truncated LH-receptor protein have been described. In 2005 a novel frameshift *insertion* in the LH-receptor gene was described *(47)*.

In this case a homozygous, one basepair insertion, T, at codon 589 resulted in a frame shift in the open reading frame, ending in a premature STOP codon 17 amino acid residues C-terminal from the insertion. The patient, a 46XY patient with complete pseudohermaphroditism, showed lack of responsiveness to LH/hCG with concomitant undetectable serum testosterone *(47)*. In view of the expected complete inactivation of the LH-receptor protein product by this truncation, the effect of the mutation was not evaluated in vitro.

Inactivating LH-receptor mutations have also been identified in the extracellular hormone-binding domain. Thus, Richter-Unruh and co-workers *(48)* identified a homozygous amino acid change, Val144Phe in exon 5 of the LH-receptor gene, in a 46XY patient with a female phenotype with clitoral enlargement and labial synechia. Upon in vitro expression, the mutant LH receptor protein was shown not to be transported to the plasma membrane. The exchange of the relatively small valine residue by the large phenylalanine probably caused incorrect folding of the mutant receptor protein, resulting in recognition by the chaperone proteins and prevention of plasma membrane transport. Such a pattern of expression, i.e., inability of the protein to be transported to the cell surface, has also been shown with some other missense or truncation mutations such as Cys343Ser, Cys543Arg *(49)*, Leu502Pro *(50)*, and Tyr612Stop *(51)*. Basic modeling of the Val144Phe mutation on the basis of a previously published model of the extracellular domain of the LH receptor *(16)* showed that Val144 is located in the fourth α helical segment on the convex face of the leucine-rich repeat domain of the extracellular LH-receptor domain. Possibly the bulky phenylalanine has steric hindrance with nearby amino acid residues, notably Phe119 *(48)*.

Another missense mutation in the LH receptor, Phe194 Val, was identified in a homozygous complete 46XY pseudohermaphrodite *(52)*. This missense mutation is located in exon 7 in a motif that is conserved in the glycoprotein hormone receptor family. Immunohistochemistry on testis of the patient showed paucity of mature Leydig cells and very low expression of the mutant Phe194Val LH receptor. In vitro expression showed absence of hCG-stimulated cAMP production by the Phe194Val LH receptor, explaining the phenotype of the patient. Phe194 is located in an interesting motif, conserved in the gonadotropin receptors: AFNGT. $N^{195}GT$ is a glycosylation motif and the change of the homologous Ala^{189} in the FSH receptor to Val, identified in some patients with hypergonadotropic ovarian dysgenesis *(53)*, causes complete inactivity of the FSH receptor due to intracellular sequestration of the mutant receptor protein *(54)*. An Asn191Ile change in the FSH receptor, which was found in a heterozygous patient without phenotype, leads to decreased coupling to the cAMP pathway *(55)*. Similar to Val144, Phe194 is also located on the convex side of the leucine-rich repeat domain (in the sixth α-helix of the extracellular hormone-binding domain), according to the same model as referred to above *(16)*.

From the same group an interesting follow-up to the del (exon 10) LH-receptor mutant has been published. In the original paper, a patient who was homozygous for a *LH receptor* mutation that caused absence of the amino acids encoded by exon 10 was described. The patient had normal male sex differentiation, but did not show signs of puberty, apparently because the del(exon10) LH receptor did not respond to LH, while it was sensitive to hCG, hence the intact male sex differentiation *(56)*. In an in vitro follow-up on this study, it was indeed shown that there is almost no difference in the ED50 of hCG for the wild-type and del (exon 10) LH receptors, whereas the dose–response relation for LH is severely shifted to the right by the exon 10 deletion *(57)*. Interestingly, in the marmoset monkey the LH receptor always misses the amino acids equivalent to the exon 10 encoded amino acid residues *(58)*. It appears that the marmoset has switched to CG for its main luteinizing hormone, because the marmoset pituitary does not express *LH*β, but rather *CG*β *(59)*. The authors postulate that, owing to an unknown mutational event in evolution, expression of marmoset LH was completely abolished, and marmoset CG—which, unlike LH, acts normally even when exon 10 is missing from the LH receptor—took over its function.

LHR Polymorphisms

The LH-receptor gene carries a large number of single nucleotide polymorphisms (SNPs). According to the SNP per website (http://SNPper.chip.org) 282 SNPs are found in the LH-receptor gene, resulting in an average distance between SNPs of 306 basepairs. The most frequent LH-receptor polymorphisms that have been identified are the absence or presence of a two-amino-acid insertion at position 18 in exon1 (insLQ), and two variable amino acids at position 291 and 312, respectively: N291S and N312S *(60)*. In addition an R124Q has been described as a SNP but with low frequency. The 291 and 312 polymorphic amino acids are located in exon 10, which, when deleted, renders the LH receptor relatively insensitive to LH while maintaining responsiveness to hCG *(57,61)*. Association studies with the latter two polymorphisms have not been reported. However, a recent study by Powell and co-workers *(31)* in a cohort of breast cancer cases revealed that, although carriers of the insLQ allele do not appear to have a greater risk for developing breast cancer, patients that are either homozygous for insLQ or are heterozygous carries of the allele have significantly worse overall survival (HR 2.4; $p = 0.006$) *(31)*. In addition, trends were observed for associations between the insLQ carriers and nodal involvement or larger tumor size. These results indicate that the insLQ polymorphic insertion probably has an effect on LH-receptor protein function, although one report showed that in vitro hCG signal transduction of the two LH-receptor variants was not different *(62)*. The finding of an association of breast cancer disease with insLQ needs independent confirmation. A plausible explanation for the observed association of insLQ

with breast cancer could be through an effect of LH through the LH receptor, with or without insLQ, causing an increase in the levels of estrogen in the serum, which in turn may stimulate breast cancer cells, and therefore cause recurrence of the disease. Alternatively, direct effects of LH on breast cancer cells themselves have been suggested *(63)*. Because LH is a major regulator of ovarian steroid hormone production, further investigations are needed into the possible role of the polymorphism in other steroid-hormone related diseases. These include prostate cancer in the male and estrogen-related disease endpoints such as bone density or fractures in elderly women.

Mutations and Polymorphisms in Human Follicle-Stimulating Hormone and its Receptor

FSHβ Subunit

Mutations

The currently known *FSH*β mutations are presented in Fig. 1. The first mutation in *FSH*β was detected in a woman suffering from primary amenorrhea and infertility *(64)*. It was a two-nucleotide deletion in codon 61 (Val) that gave rise to completely altered amino acids 61–86, followed by premature stop codon and lack of translation of amino acids 87–111. The phenotypes caused by FSH inactivation are quite expected in view of the knowledge about functions of this hormone. However, they have revealed certain interesting details that elucidate further some poorly known aspects of FSH function. The women have sexual infantilism and infertility because of lack of follicular maturation. The three men with *FSH*β mutation are all normally masculinized but azoospermic *(3,65,66)*. This phenotype is at variance with the *FSH-receptor* mutation as will be discussed below.

The number of individuals known to be hypogonadal due to *FSH*β mutation is currently seven, four women and three men, and a total of four different mutations have been identified: Cys51Gly, Val61Δ2bp/87X, Tyr76X, and Cys82Arg *(1,3)*. It is typical of all of these mutations that they interfere with the cystine knot structure of the peptide, which is essential for disulfide bonds and tertiary structure, dimerization with α-subunit, and acquisition of biological activity. The deletion mutants, in addition, lack the "cystine noose" necessary for receptor recognition, and the seat belt needed to stabilize the α/β dimer. The cystine knot is disturbed either by mutation of critical cysteines to other amino acids or by premature stop codon through deletion and frame shift. Hence, from the molecular point of view, the current knowledge about the genetic and crystalline structure of FSH provides sufficient background information to explain the hormone inactivation at molecular level. Despite the partially preserved phenotype, no residual bioactivity could be found in the most recently discovered Tyr76X mutation *(3)*, when compared with the other mutations causing more complete phenotypes. The explanation of this finding remains open, but it suggests that even milder mutations or polymorphisms of *FSH*β could exist, causing ovulation disorders in women and oligozoospermia in men.

All four women with inactivating *FSH*β mutations have largely similar phenotypes, which in the complete form includes absent puberty and infertility due to lack of follicular maturation. Other features are the absence of breast development, primary amenorrhea, low estrogen production, undetectable serum FSH, and increased LH. As expected, a large number of undeveloped follicles, usually in the primordial stage but sometimes more advanced, can be found in the ovaries of the affected individuals. The two previously characterized men had azoospermia, one had normal puberty and puberty was delayed in the other.

The latest mutation, Tyr76X, was found in two affected siblings, a male and a female. Both had unmeasurable FSH by immunoassay and bioassay. The female sibling presented with a partial phenotype of partial puberty with stage II-III breast development, primary amenorrhea, and low estradiol. The man had complete puberty with normal serum testosterone level, but azoospermia with lightly reduced testes size (12 cm^2). Testicular biopsy of the man revealed azoospermia, fibrosis, and increased Leydig cell volume density.

Polymorphisms

Somewhat surprisingly, and in contrast to *LH*β, the *FSH*β subunit appears to be highly conserved. Only a few silent polymorphisms and totally conserved promoter region were found in the *FSH*β gene when studied recently in 50 Danish and 50 Finnish DNA samples (T. Lamminen and I. T. Huhtaniemi, unpublished observation).

FSH Receptor

As the mutations of LH receptor, those of FSH receptor exist in principle in activating and inactivating form (Fig. 3). The number of inactivating mutations is slowly increasing, but with one single exception of persistent spermatogenesis of a hypophysectomized man *(67)*, other activating mutations of the FSH receptor, especially in women, have not been detected. The following paragraph summarizes recent discoveries in this topic.

Activating Mutations

The only apparently activating mutation in the *FSH receptor* was that described by Gromoll et al. *(67)*. The patient was a previously hypophysectomized man who maintained normal spermatogenesis in spite of undetectable gonadotropins. It is somewhat surprising that no other mutations have been detected in nearly 10 yr. Such mutations are, however, possible as has been shown with site-directed mutagenesis in vitro *(68)*, but whether they cause a phenotype in vivo has not yet been resolved. Search for activating *FSHR* mutations in candidate diseases, such as premature ovarian failure, ovarian tumors, megalotestes, precocious puberty, and twin pregnancies, has been unsuccessful *(42,69–73)*. It remains a possibility, therefore, that activating *FSHR* muta-

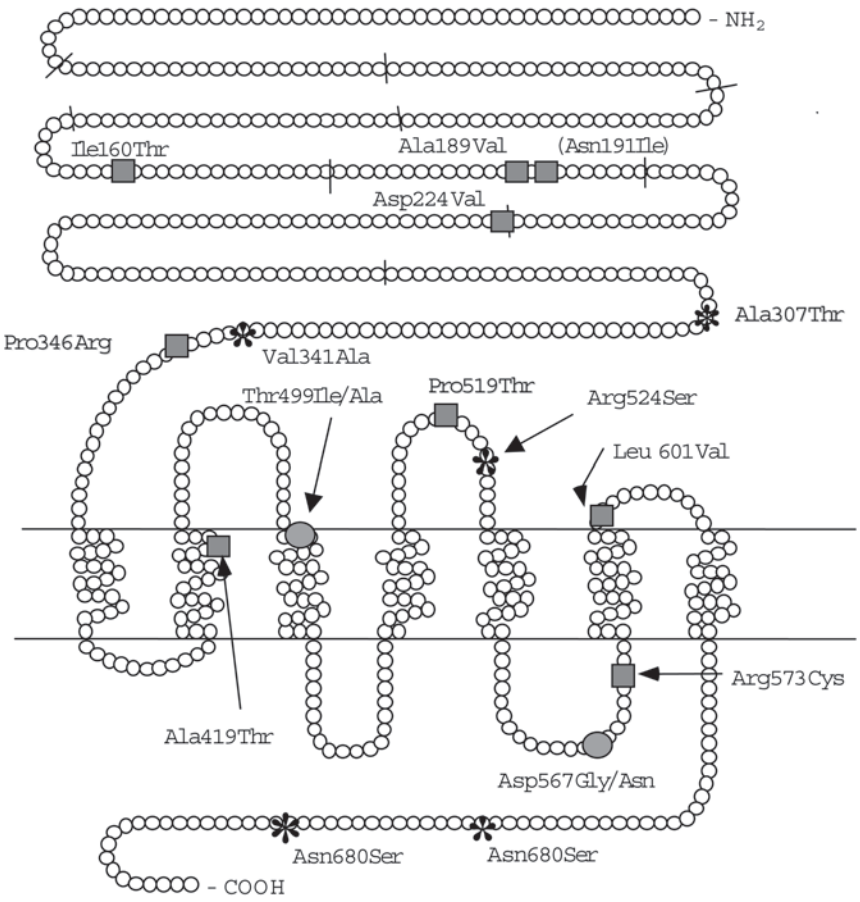

Fig. 3. The currently known mutations and amino-acid-altering polymorphisms in the human FSH receptor gene. The circles depict the activating, the squares inactivation mutations, and the asterisks the polymorphisms.

tion has no phenotype or that it differs totally from our educated guesses. Another explanation is that the phenotype can only be observed in extreme situations, such as after hypophysectomy *(67)*. An animal model for activating *FSH-receptor* mutation would be seminal in resolving this intriguing question.

Two very interesting cases of recurrent, spontaneous pregnancy-associated ovarian hyperstimulation syndrome (OHSS) were recently reported *(74–76)*. In both of them, a mutation of the *FSH receptor* was found that relaxes the ligand specificity of the receptor in such a way that it also binds and becomes activated by hCG. In one of the cases *(74)*, the woman developed the syndrome during four of her five pregnancies, and also her sisters had had pregnancy-associated OHSS. In the second case *(75)*, a similar condition developed during all four pregnancies of the woman. Heterozygous Thr449Ile and Asp567Asn mutations were found in the two patients, respectively. Subsequently, a third similar patient was found with Thr499Ala mutation in the *FSH receptor (77)*. In vitro studies with the mutated receptors demonstrated in each case that high concentrations of hCG were able to induce cAMP production of cells transfected with the mutant receptor. In two of the cases, low level of constitutive receptor activation and response of the mutated receptor to TSH was also found *(75,77)*. Both mutations were, unexpectedly, not in the ligand-binding extracellular domain but in the transmembrane region, participating in signal transduction. Because no high-affinity hCG binding to the mutated receptor could be demonstrated, hCG probably interacts at low affinity with the transmembrane region sufficiently to shift its conformation to the active form. Another possibility is that hCG binds at low affinity to the FSH-receptor ectodomain, and if the mutation of the transmembrane domain reduces its activation barriers, partial activation of signaling would be possible *(78)*. The pathogenesis of the syndrome can thus be explained by "promiscuous" stimulation of follicles by hCG, resulting in excessive follicular recruitment. When such follicles grow, they also apparently acquire LH-receptor expression and are therefore stimulated through activation of these receptors as well. Spontaneous OHSS occurs between wk 6 and 10 of pregnancy, which coincides with the highest hCG concentrations in maternal circulation *(79)*. In normal women, this occurs only when hCG levels are abnormally high, resulting in sufficient binding of hCG to the normal FSH receptor.

Inactivating Mutations

Altogether nine inactivating mutations are known today in the human *FSH receptor*. The first one, Ala189Val, described about 10 yr ago in Finland *(53)*, is still the only one detected in multiple families. These subjects had a phenotype of hypergonadotropic hypogonadism, primary or early onset secondary amenorrhea, variable development of secondary sex characteristics, and arrest of follicular maturation between primordial and preantral stage. The mutation belongs to the Finnish heritage of genetic diseases *(80)*, and despite multiple attempts the same mutation has not been found in any other population. Newer studies on this mutation have shown that the mutated receptor protein becomes sequestered near-totally within the cells *(54)*, explaining the functional consequence of the mutation. When the mutated receptor was overexpressed in COS-7 cells, low cAMP response could be measured, but the IP3 response was totally lost. And when two women and one man homozygous for the same mutation were treated with massive doses of recombinant FSH (300–900 IU/d), no functional responses of the ovaries or testes were observed *(81)*. Therefore, this mutation must be considered from the functional point of view totally inactivating. It is curious that a conserved Ala to Val mutation causes such a dramatic alteration in behavior of the receptor protein. This indicates that the FSH receptor is very sensitive to structural alterations that interfere easily with trafficking of the protein causing its intracellular sequestration. Similar observations have been made upon site-directed mutagenesis studies of the FSH receptor.

Apart from the Finnish mutation, the other patients detected earlier have been compound heterozygotes of totally and partially inactivating *FSH-receptor* mutations, and therefore their phenotypes have been less prominent, with secondary amenorrhea, gonadotropin resistance, normal ovarian size, and presence of follicles up to the antral stage *(82,83)*. Several additional *FSH-receptor* mutations have been recently discovered, conspicuously all in women. As was demonstrated with the Ala189Val mutation, the male phenotype of FSH-receptor inactivation is rather unassuming, with normal androgen production, poor sperm quality, but possibility to maintain fertility *(84)*. It is unlikely that inactivating FSH-receptor mutations will be discovered in men other than those belonging to families where a mutation has been discovered through the prominent female phenotype.

A new Finnish patient with a compound heterozygous mutation of Ala189Val and Ala419Thr was recently reported *(85)*. The subject was investigated at 17 yr of age because of primary amenorrhea. She had normal secondary sex characteristics, very low estrogen levels but clear signs of endometrial estrogen stimulation in transvaginal ultrasound investigation, and normal progestin challenge test. Hence, the phenotype was less severe than with the Ala189Val mutation. The mutation was found to have minimal effect on ligand binding, but it totally abolished the FSH signaling. A new case of completely inactivating *FSHR* mutation was recently described *(86)*. The affected female presented with hypergonadotropic premature ovarian failure, very low estrogen and inhibin B levels, and total lack of response to high doses of recombinant FSH. The subject had a homozygous Pro419Thr mutation, which is localized in a conserved motif of the gonadotropin and TSH receptors in the second extracellular loop of the transmembrane region. As is typical for inactivating *FSH-receptor* mutations, the mutated receptor protein was entrapped intracellularly and was consequently unable to bind hormone and evoke signaling. Histological analysis of ovarian biopsy confirmed the finding of the Finnish mutation on arrest of follicular maturation beyond the primary stage. The last mutation reported was a Pro348Arg substitution in the extracellular part of exon 10 in a hypergonadotrophic woman with delayed puberty and primary amenorrhea *(87)*. This mutation totally abolished ligand binding, but it was not studied whether it was due to intracellular sequestration of the receptor or to genuine lack of ligand binding to an otherwise normally exteriorized receptor protein.

Despite the rarity of the currently known *FSHR* mutations, there is good correlation between the phenotype and the degree of receptor inactivation, as well as the site of mutation and its functional consequences, in the same fashion as with the larger number of *LHR* mutations *(1)*. All mutations in the extracellular region, 1le160Thr, Ala189Val, Asn191Ile, Asp224Val, and Pro346Arg, cause a defect in targeting of the protein to the cell membrane, and therefore they lack both ligand binding and signaling. In contrast, the three mutations in the transmembrane region, Ala419Thr, Arg573Cys, and Leu602Val, have minimal effect on ligand binding but impair to various extents, but not totally, the signal transduction. The only exception is the Pro519Thr mutation, present in the second exoloop of the transmembrane region, which is devoid of ligand binding and signaling. It thus seems that the location, rather than nature of amino acid alteration, determines the functional response. The mutations in the transmembrane region, in addition, seem to cause less total receptor inactivation. It is important to know that the ovaries of patients with the milder forms of mutations may respond to high-dose FSH stimulation, whereas no response was found with the complete form of receptor inactivation. Hence, the molecular diagnosis of these rare patients may help in design of rational treatment for their infertility. All heterozygotes for the mutations so far studied have been free from phenotype, which indicated that one functional *FSH-receptor* allele is sufficient for normal reproductive function.

Polymorphisms

In addition to the inactivating and activating mutations, several common single nucleotide polymorphisms (SNPs)

exist in the human *FSH-receptor* gene (http://www.ncbi.nlm.nih.gov/SNP/). The number is large, >700, but it is explained by the large size of the *FSH-receptor* gene (191 kb), and the average SNP distance (282) is similar with that of *LH-receptor* (307). The majority of the polymorphisms are intronic, five are located in the *FSH-receptor* coding region and one in the promoter. The exonic polymorphisms, curiously, are all in exon 10, at locations 307, 329, 524, 665, and 680. The amino acid alterations brought about are Ala 307Thr, Arg524Ser, Ala665Thr, and Ser680Asn; the 392 SNP is silent.

The two commonest SNPs in *FSH receptor* are the Ala 307Thr and Ser680Asn polymorphisms, which have been found to be in linkage disequilibrium in most of the studies, and the two allelic variants Thr307/Asn680 and Ala307/Ser680 are almost equally distributed in Caucasian populations *(88)*; the other combinations of these SNPs represent less than 5% on the *FSH-receptor* alleles. It has been found in several studies that the amount of FSH needed for ovarian hyperstimulation upon IVF cycles, to achieve the same estradiol level, was lower in women with the Asn/Asn variant at 680 than the other two combinations Ser/Ser and Asn/Ser, indicating that the Ser680 allele has lower sensitivity to ligand stimulation *(89–92)*. In a study on correlation of the *FSH-receptor* genotype with OHSS *(93)*, it was found that the Ser680 allele was enriched in control IVF population, and the OHSS population had even higher enrichment of this allele in comparison to controls (57 vs 39%). This result was unexpected and difficult to reconcile with the poor FSH response of the Ser680 allele documented in the previous studies. Furthermore, the Asn680 allele was enriched among the severe forms of OHSS. The conclusion of this study was that the *FSH-receptor* genotype cannot predict the development of OHSS, but may be a predictor of severe cases.

Some studies have shown association of the Ser680 polymorphism and amenorrhea or anovulation *(90,94)* but no association with premature ovarian failure has been found *(69,95)*, and the association of these polymorphisms with polycystic ovarian syndrome has yielded conflicting results in different populations *(72,90,95)*. Neither is there association with twinning and the *FSH-receptor* allele *(96)*, and no relationship to male fertility parameters has been found *(97,98)*. The two 680 FSHR alleles seem to have similar biological activities in vitro *(90,98)* which leaves the mechanism of the aforementioned clinical correlations open. Altogether, as is typical of polymorphisms, the phenotypic effects appear marginal and show large differences between populations.

Concluding Remarks

In the near future association studies of polymorphic gonadotropin and receptor variants with population disease endpoints will yield more information on the general role of these important hormones in human physiology. We are looking forward to these studies because they may also shine light on the discussion in the field about the role of extragonadal expression of the gonadotropin receptor, especially the LH receptor. However, studies in animal models are essential to finalize this discussion.

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